## **ABSTRACT**

The invention provides a method for quantifying an initial ratio of the amounts of at least two nucleic acids of interest in a sample by means of a multiplex nucleic acid amplification reaction, comprising amplifying the nucleic acids of interest in the amplification reaction, measuring the amount of at least two nucleic acids of interest at at least two different time points in the reaction, determining from at least two of the measurements the amplification rate of the at least two nucleic acids of interest, comparing the rates with a reference, and determining from the comparison the initial ratio of the amounts of the at least two nucleic acids of interest in the sample. Preferably, at least one variable factor in the nucleic acid amplification reaction is adjusted in order to allow detectable levels of all nucleic acids of interest to be reached before an amplification and/or detection limit of one or more of the nucleic acids of interest is reached. With the invention, small differences between ratios can be determined, as can direct measurement of the initial nucleic acid ratio. The invention is suitable for determining functioning of a cellular organism, the staging of a disease, and therapeutic activity and/or possible side effects of a compound.

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